The molecular basis of algal defense

As a measure against the adaptive potential of enemies and for reduction of metabolic costs, defense in multicellular organisms is often regulated. The regulation usually involves molecular perception of enemy presence or activity, followed by activation or induction – either local or systemic - of defense-related proteins. Animals and vascular plants are known since more than a century to defend themselves facultatively against pathogens and grazers. Macroalgal defense, in contrast, has until recently mainly been regarded as "constitutive" in the sense of "permanent" or "unregulated". Indeed, many macroalgae appear to be chemically defended at constantly high levels and this is possibly one of the reasons why the first evidence of enemy-aroused resistance in a macroalga was only detected a few years ago.

For example, the bladder wrack *Fucus vesiculosus* has been shown at IFM-GEOMAR to respond with reduced palatability when it is exposed to herbivorous animals. This brown alga, which is widely distributed in the intertidal North Atlantic and one of the most important habitat forming seaweeds in the Baltic Sea, responds in different ways to distinct consumers. The herbivore *Idotea balthica* induces reduced palatability in grazed *Fucus*, as well as in ungrazed individuals in the neighbourhood of grazed specimens. A water soluble molecular signal obviously indicates the prevalence of *Idotea* grazing within *Fucus* populations. In contrast, the herbivorous snail *Littorina littorea* only reduces palatability in individuals that have been directly grazed and simple mechanical wounding of *Fucus* induces no defensive response. Thus, the bladder wrack differentiates not only between physical damage and natural herbivory, but also between different grazers. Bioassay-guided purification of algal extracts has recently revealed that several different unpalatable metabolites accumulate in *Idotea*-grazed *Fucus*. Some of these compounds are polyphenolics, while the chemical nature of others is still under investigation.

Further evidence of enemy-induced defense comes from the kelp *Saccharina latissima*, another inhabitant of cold temperate waters. Different from populations in the North Sea, *Saccharina* sporophytes in the western Baltic are seasonal, developing during winter and perishing in summer, when water temperatures may raise to 20°C or more. The cell wall matrix of *Saccharina* consists of alginic acid, a polysaccharide composed of guluronic acid and mannuronic acid. On the algal surface alginic acid is constantly subject to more or less intense microbial degradation, which results in a release of oligoalginates. Oligoalginates that are rich in guluronic acid are perceived by *Saccharina*. They activate immediate responses such as an oxidative burst - an enzymatic production of reactive oxygen species like hydrogen peroxide (Fig. 1). These are released into the cell wall free space and the surrounding water, where they drive enzymatic processes such as haloperoxidation. Reactive oxygen species – either alone or in combination with peroxidation products – may directly inhibit certain alginate degraders. In addition, they are suspected to act as secondary signals within the cell, regulating the transcription of defense-related proteins. A study conducted in 2007 at IFM-GEOMAR has shown that a complete loss of the algal capacity to respond to oligoguluronate precedes its seasonal death in early summer, at a time when photosynthesis is still functional. An incapacity for defense against opportunistic pathogens therefore appears as one of the reasons for the death.

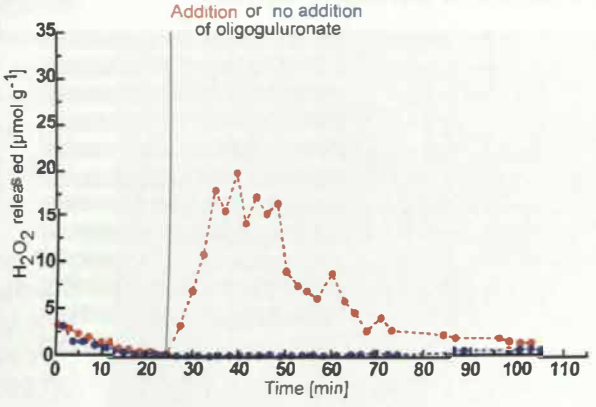

Fig. 1: Release of hydrogen peroxide into the medium by Saccharina latissima exposed and unexposed to oligo-guluronic acid.

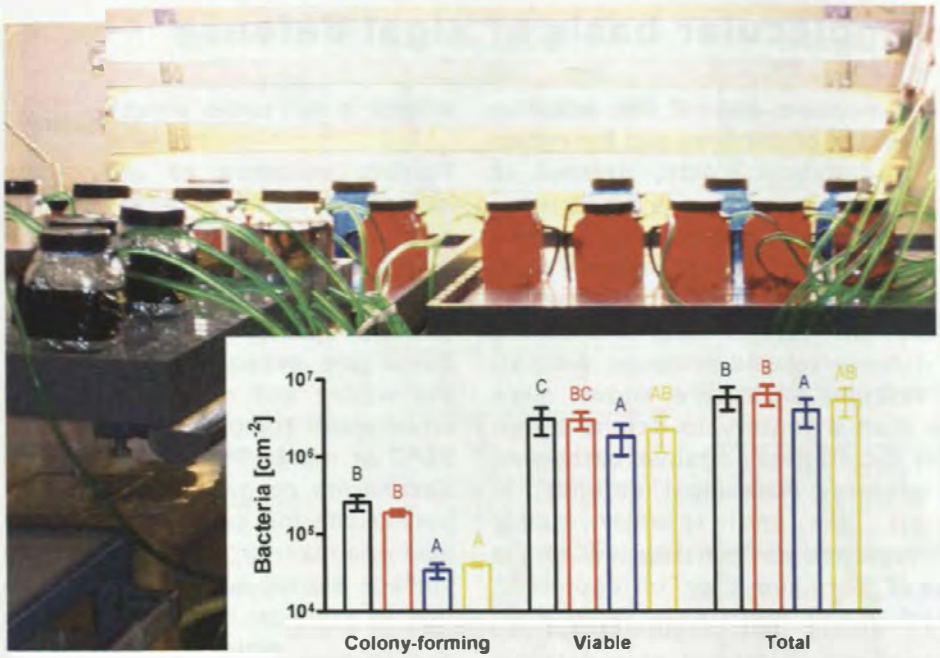

Fig. 2: Numers of colony forming, viable and total bacteria on Fucus vesiculosus incubated for 16 h in darkness, followed by 7 h of incubation in darkness or exposed to 20 µmol m^{-2} s^{-1} of red, blue or white light. Average +/- SE, n = 5. Data marked by different letters are significantly different (Tukey-test, α = 0.05).

Evidence of enemy-induced defense against microorganisms has not been detected in *Fucus* yet. Instead, recent results indicate that blue light controls rapid variations in the defense of this alga against microorganisms. In natural stands, the numbers of bacteria associated with *Fucus* – but not those associated with non-living surfaces - oscillate in a diurnal cycle. Marked decreases of bacterial numbers have been observed during the first hours after sun rise, followed by a rise in bacterial abundance during the following hours. Comparable oscillations were also observed in the laboratory, provided that *F. vesiculosus* was exposed to relatively low irradiances (20 µmol photons m^{-2} s^{-1}) of blue or white light. In contrast, red light exposure and continuous absence of light for more than the usual darkness period did not result in such an effect, and associated microorganisms reached higher numbers than usual (Fig. 2). Regulation of antimicrobial defense in *Fucus* seems therefore to be based in part upon blue light-perception, which apparently triggers pulses of defensive activity.

First hints about the possible nature of this activity come from an other study that is currently conducted at IFM-GEOMAR in cooperation with the microbiology department of Kiel University. This project deals with the identification of algal metabolites that control the overall composition of bacterial biofilms. In order to simulate the excretion of secondary metabolites by seaweeds a device has been developed that allows it to release metabolites at constant pace and over several days through a polysaccharide matrix. The matrix resembles algal cell walls chemically and mechanically. It may be exposed in the marine environment and biofilms that develop on it can be sampled and analyzed. Release of *Fucus* surface extracts from this device results in the development of less dense and less diverse (Fig. 3) biofilms, indicating that *Fucus* has the capacity to control its associated bacterial flora through excretion of specific secondary metabolites.

A newly launched project aims to design a transcript profiling tool for *Fucus* and to identify gene transcripts that are up- or downregulated during enemy attacks. This approach is expected to further improve our understanding of the molecular basis of algal defense.

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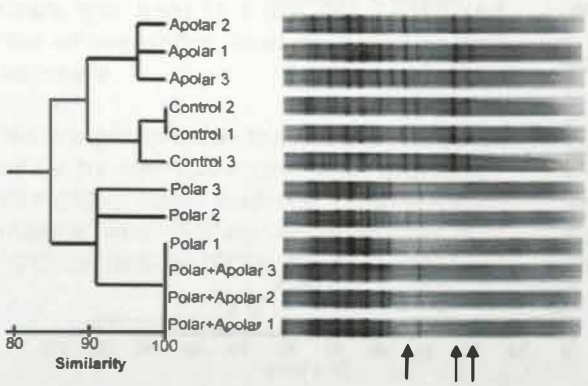

Fig. 3: Similarity of epibacterial communities on phytagel after several days of simulated excretion of polar, apolar or no (control) Fucus vesiculosus surface extract. 16S rDNA was extracted from the epibacterial communities, amplified and separated by denaturing gradient gel electrophoresis. Arrows indicate bands that are missing after treatment with polar extract, representing missing bacterial species.